Claims

1. Method for preparing, in a prokaryotic cell, a recombinant viral vector derived from a parent virus into the genome of which an exogenous DNA sequence is inserted, by intermolecular recombination between (i) a first DNA fragment comprising all or part of said genome of the parent virus and (ii) a second DNA fragment comprising said exogenous DNA sequence surrounded by flanking sequences A and B which are homologous to (i).

2. Method according to claim 1, characterized in that the parent virus is selected from the group consisting of adenoviruses, retroviruses, adeno-associated viruses, poxviruses and herpesviruses.

3. Method according to claim 2, characterized in that the parent virus is an adenovirus of human, canine, avian, bovine, murine, ovine, porcine or simian origin, or alternatively a hybrid adenovirus.

4. Method according to claim 3, characterized in that the parent virus is a type CAV-2 adenovirus of canine origin.

5. Method according to claim 3, characterized in that the parent virus is a serotype C adenovirus of human origin.

6. Method according to claim 5, characterized in that the parent virus is a type 5 adenovirus of human origin.

7. Method according to one of claims 1 to 6, characterized in that said exogenous DNA sequence codes for a polypeptide of therapeutic interest selected from the group consisting of coagulation factors, growth hormones, cytokines, lymphokines, tumor-suppressing polypeptides, cell receptors, ligands for cell receptors, protease inhibitors, antibodies, toxins, immunotoxins, dystrophin and polypeptides participating in cellular ion channels, such as CFTR protein.

8. Method according to one of claims 1 to 7, characterized in that the homologous flanking sequences A and B are from 10 bp to 10 kb, advantageously from 20 bp to

5 kb, preferably from 30 bp to 2 kb, and as an absolute preference from 40 bp to 1 kb, in length.

9. Method according to one of claims 1 to 8, characterized in that the first DNA fragment is linearized in the insertion region of the exogenous sequence.

10. Method according to one of claims 1 to 9, for the preparation of a recombinant viral vector which is defective for replication.

11. Method according to claim 10, for the preparation of a recombinant adenoviral vector lacking all or part of at least one region essential for replication, selected from the E1, E2 and E4 regions.

12. Method according to claim 11, characterized in that the recombinant adenoviral vector lacks, in addition, all or part of the E3 region.

13. Method according to one of claims 1 to 12, for the preparation of a recombinant viral vector of at least 20 kb.

14. Method according to claim 13, for the preparation of a recombinant viral vector of at least 30 kb.

15. Method according to claims 1 to 14, by intermolecular recombination between (i) a first DNA fragment comprising all or part of said genome of the parent virus, (ii) a second DNA fragment comprising a first portion of said DNA sequence of interest equipped at its 5′ end with said flanking sequences [sic] A and (iii) a third DNA fragment comprising a second portion of said DNA sequence of interest equipped at its 3′ end with said flanking sequences [sic] B; said second and third DNA fragments containing a homologous sequence at their respective 3′ and 5′ ends.

16. Method according to one of claims 1 to 15, for introducing a modification by deletion, mutation and/or substitution of one or more nucleotides or an exogenous DNA sequence into a viral genome.

17. Method according to one of claims 1 to 16, characterized in that said prokaryotic cell is derived from a recBC sbcBC strain of Escherichia coli.

18. Method for preparing an infectious viral particle

containing a recombinant viral vector obtained by carrying out a method according to one of claims 1 to 17, according to which:

(a) said recombinant viral vector is introduced into a mammalian cell to generate a transfected mammalian cell,

(b) said transfected mammalian cell is cultured under suitable conditions to permit the production of said viral particle, and

(c) said viral particle is recovered from the cell culture obtained in step (b).

19. Use of an infectious viral particle prepared according to claim 18 or of a recombinant viral vector prepared according to one of claims 1 to 17, for the therapeutic or surgical treatment of the human body.

20. Use according to claim 19, for the therapeutic or surgical treatment of the human body by gene therapy.

21. Pharmaceutical composition comprising a therapeutically effective amount of an infectious viral particle prepared according to claim 18 or of a viral vector prepared according to one of claims 1 to 17, in combination with a vehicle which is acceptable from a pharmaceutical standpoint.

22. Use of an infectious viral particle prepared according to claim 18 or of a recombinant viral vector prepared according to one of claims 1 to 17, for the expression of a DNA sequence of interest in a cell system.

23. Use of a recBC sbcBC strain of *E. coli* for the cloning of DNA fragments into a plasmid vector by intermolecular homologous recombination.